Genomic techniques for assessing microbial diversity in Arctic wastewater systems

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The big picture of microbial ecology

- Investigate the relationship between microbial communities and their habitat
  - Earth microbiome project (2010) (Wastewater, water, soil, sediment, air, etc.)

- To understand this, laboratory and genomic techniques are used to characterize the community and detect pathogens :
  - Microscopic and cultivation-depended (Plate count method)
  - Cultivation-independent (Marker genes, Metagenomes, and Metatranscriptomes, qPCR, RT-qPCR)

Why use cultivation-independent techniques?

- The unculturability of plate count methods: “only a small fraction of less than 1% of the cells observed by microscopy can be recovered as colonies on standard laboratory media” (Amann 2000).

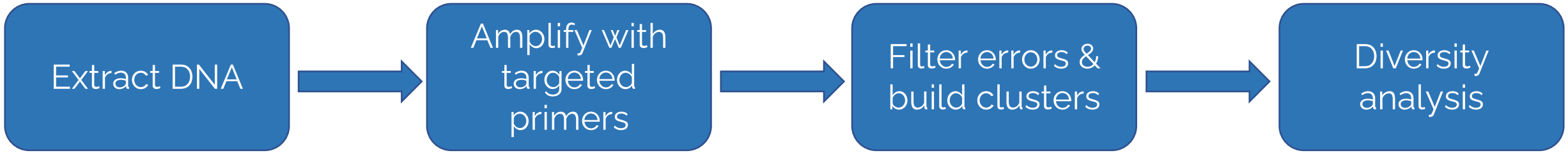
- Most of the cultured microorganisms are of minor importance while in contrast the uncultured bacteria play an essential role for most key processes in wastewater treatment plants (WWTPs) (reviewed in Loy et al. 2003).

- In the past ten years, cultivation-independent approaches have increasingly been used to study bacterial communities and detect pathogens in WWTPs.

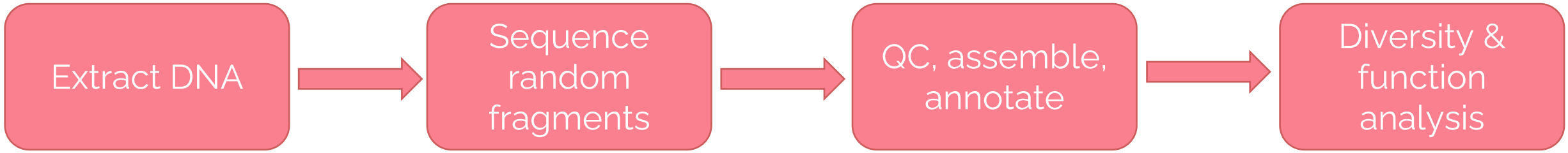
Source: Amann, R. 2000. Who is out there? Microbial aspects of diversity. Syst. Appl. Microbiol. 23: 1-8.
Loy, A., H. Daims, and M. Wagner. 2003. “Activated Sludge and Biofilms: Molecular Techniques for Determining Community Composition.” Encyclopedia of Environmental Microbiology. doi:10.1002/0471263397.env218.

Common next-generation sequencing methods for environmental metagenomics studies

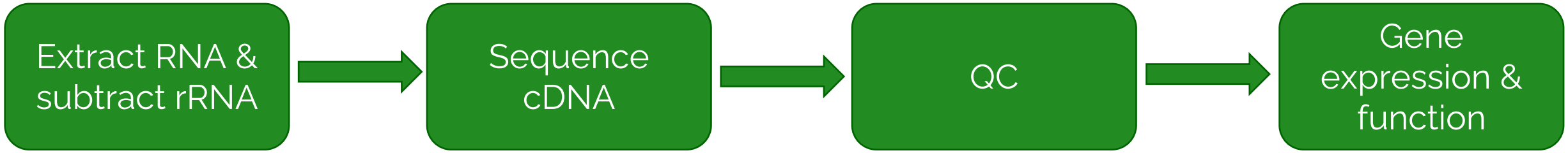
- **Marker gene**



- **Metagenomes**

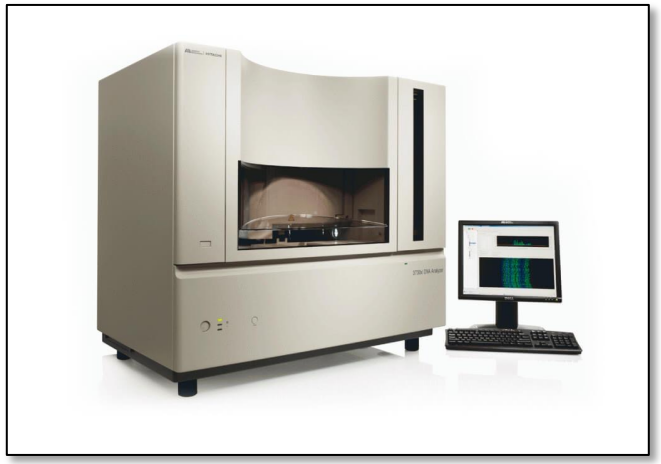


- **Metatranscriptomes**



Sequencing techniques

First generation



1977: Sanger sequencing
<1 kilobases per run, 1-3 hours
$2 per run
Small pieces (500 – 1000 bp)

Second generation

2011: 454, Illumina Miseq
15 gigabases per run, 1 day
$1000 – 1500 per run
Smaller pieces (150 – 400 bp)

Third generation

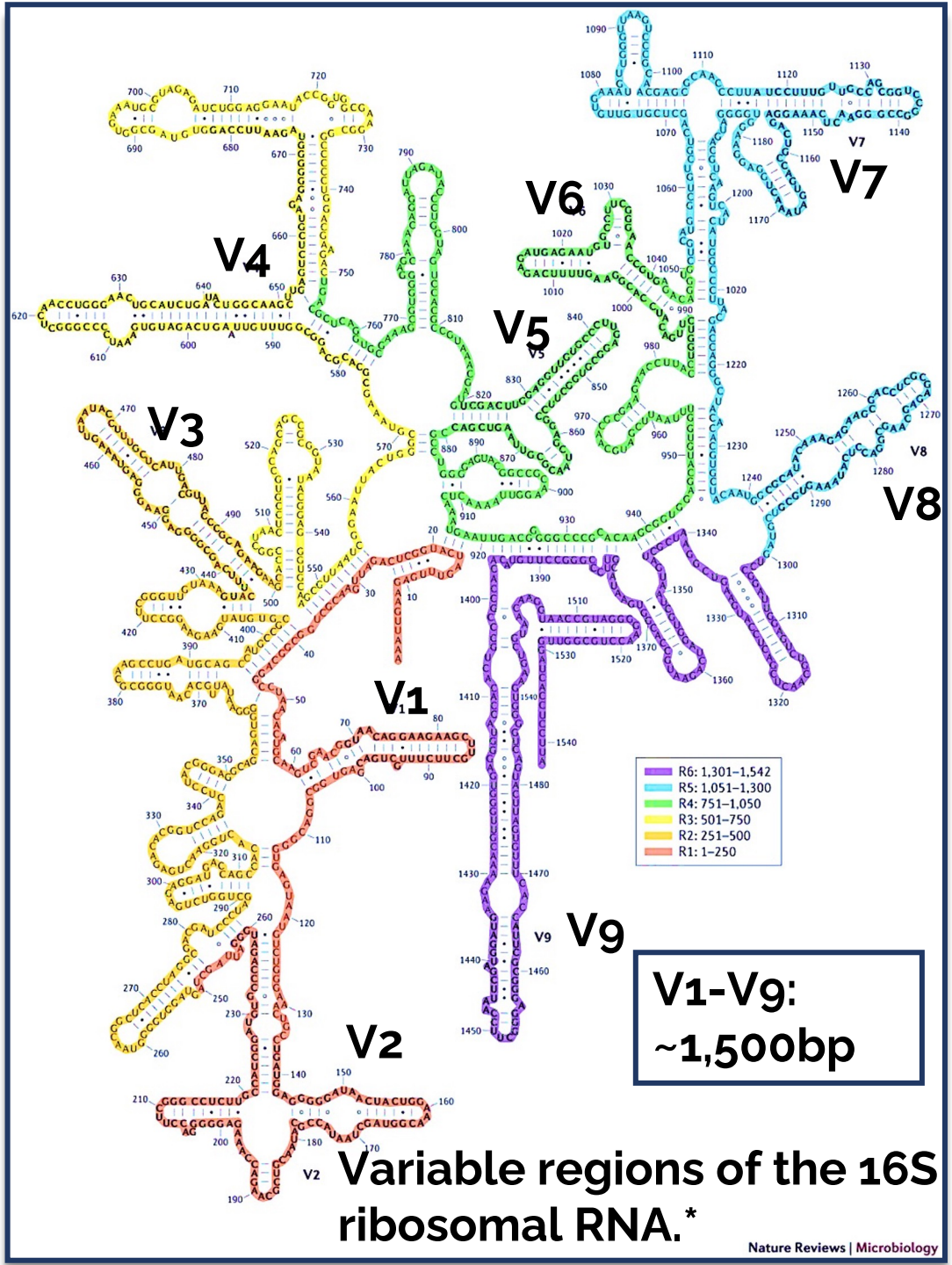
2015: Oxford Nanopore MinION, PacBio,
Nanopore: portable, weight: <100g, length: <10 cm, up to 50 gigabases per run
72 hours, commercially available – $1000
Huge pieces (max so far, 200 – 300 kb)

Short-read sequencing

Long-read sequencing

• Why use the *16S rRNA* gene in marker gene sequencing methods ?

- Present in all living organisms
- Highly conserved and variable regions
- Huge reference databases, *16S rRNA* gene (1,500 bp) most commonly used
- Behaves like a molecular clock – the universal phylogenetic marker

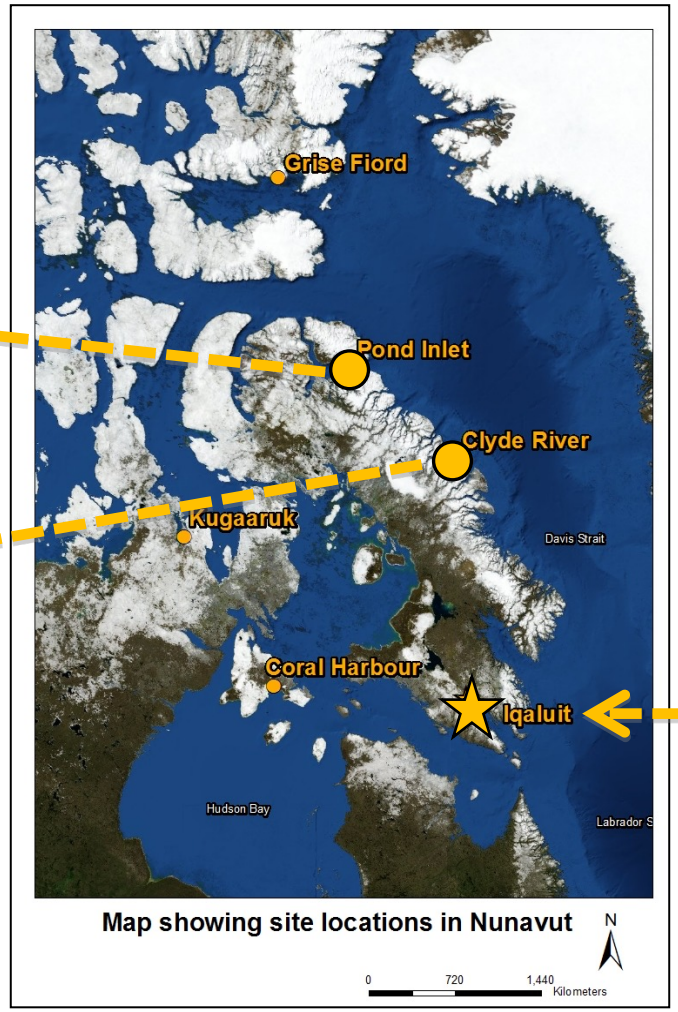


Source: * Yarza, Pablo, Pelin Yilmaz, Elmar Pruesse, Frank Oliver Glöckner, Wolfgang Ludwig, Karl-Heinz Schleifer, William B. Whitman, Jean Euzéby, Rudolf Amann, and Ramon Rosselló-Móra. 2014. "Uniting the Classification of Cultured and Uncultured Bacteria and Archaea Using 16S rRNA Gene Sequences." *Nature Reviews Microbiology* 12 (9): 635–45. doi:10.1038/nrmicro3330.

Study Sites

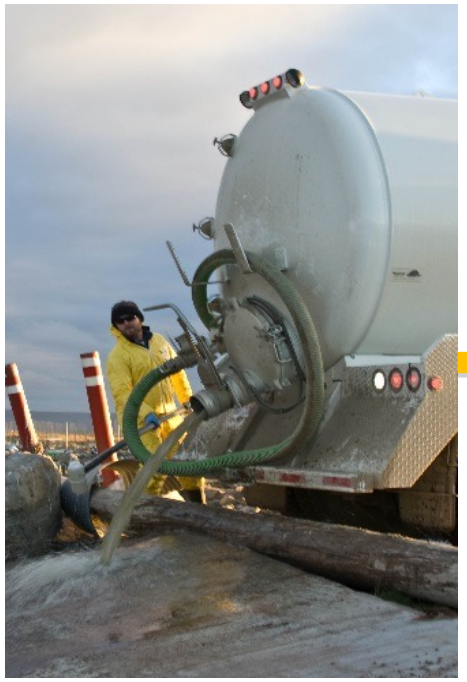
Pond Inlet

Clyde River



2012-2014 study sites

Northern Water Quality Laboratory in Iqaluit



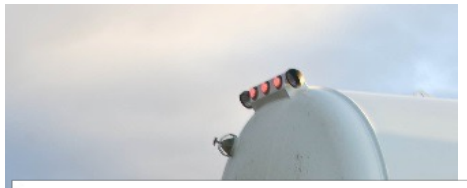
Trucks: Raw wastewater

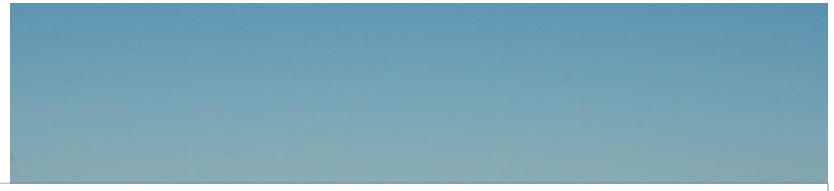
WSP

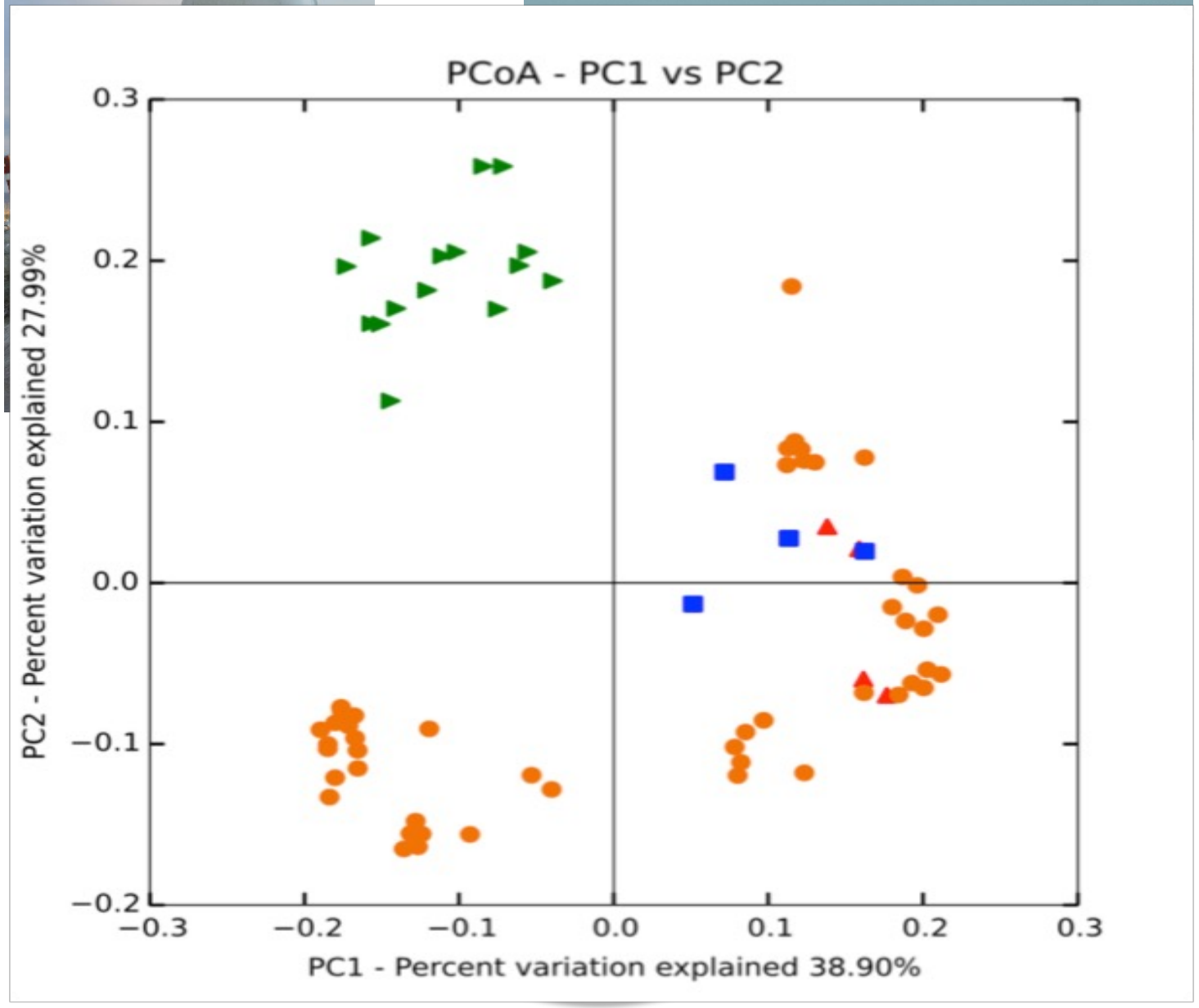
Effluents

Outfalls

An example of microbial communities affected by Pond Inlet WSP treatment train







Raw

WSP

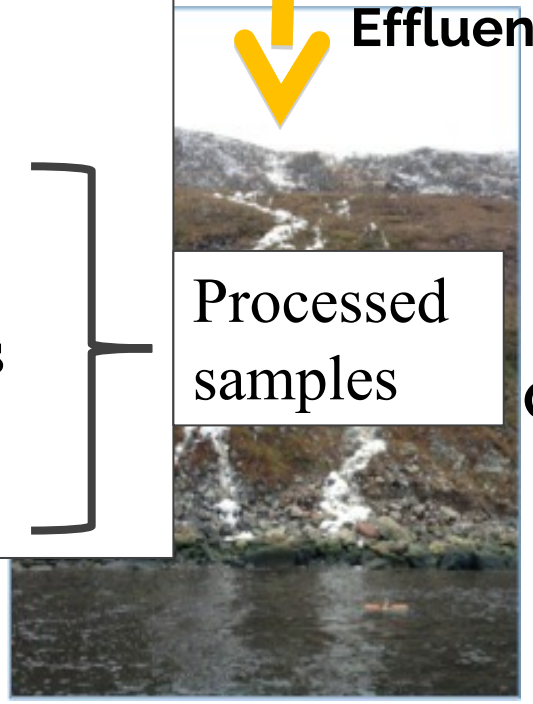
Effluents

Outfalls

Processed samples

Effluents

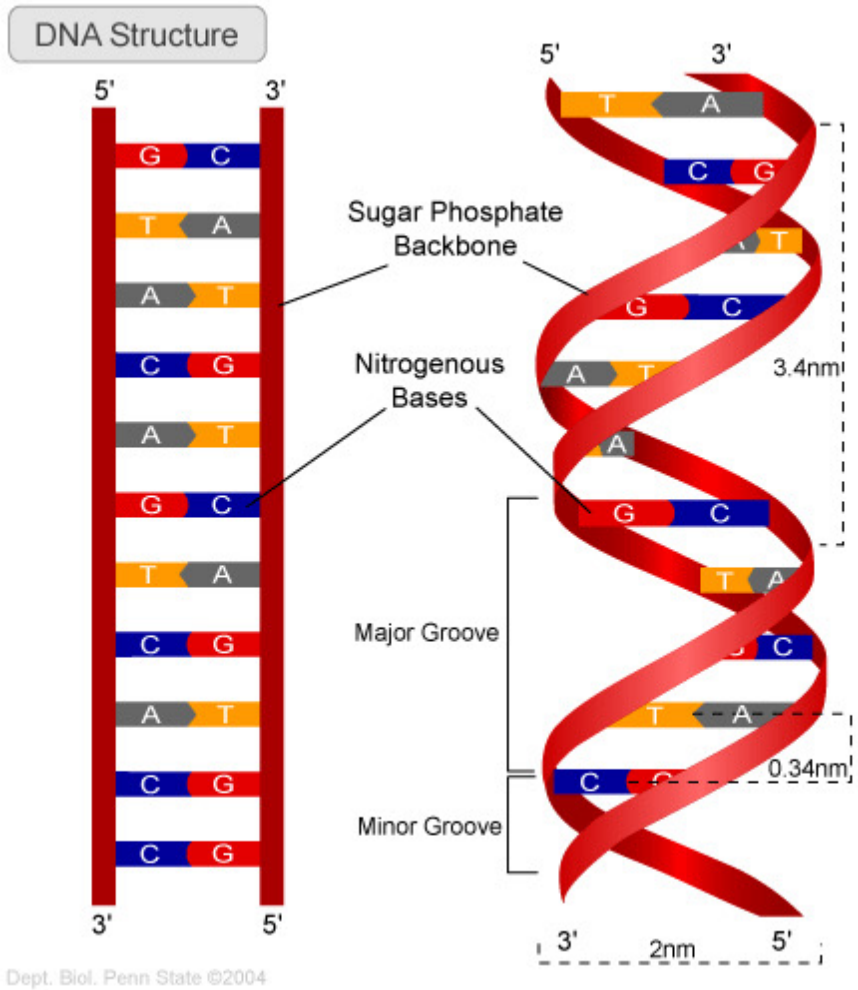
Outfalls

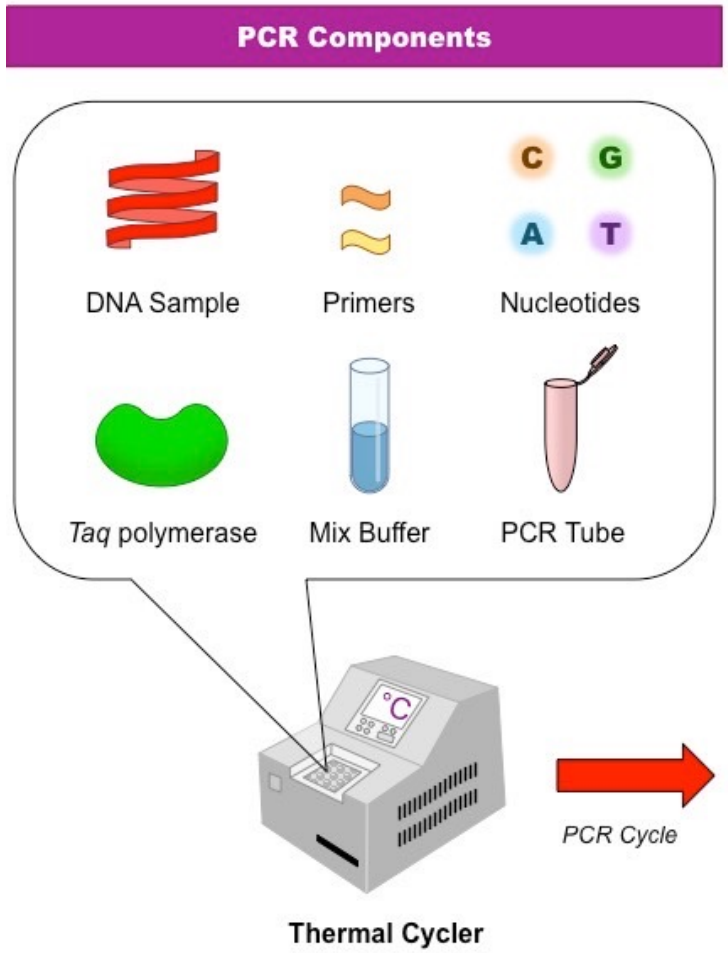


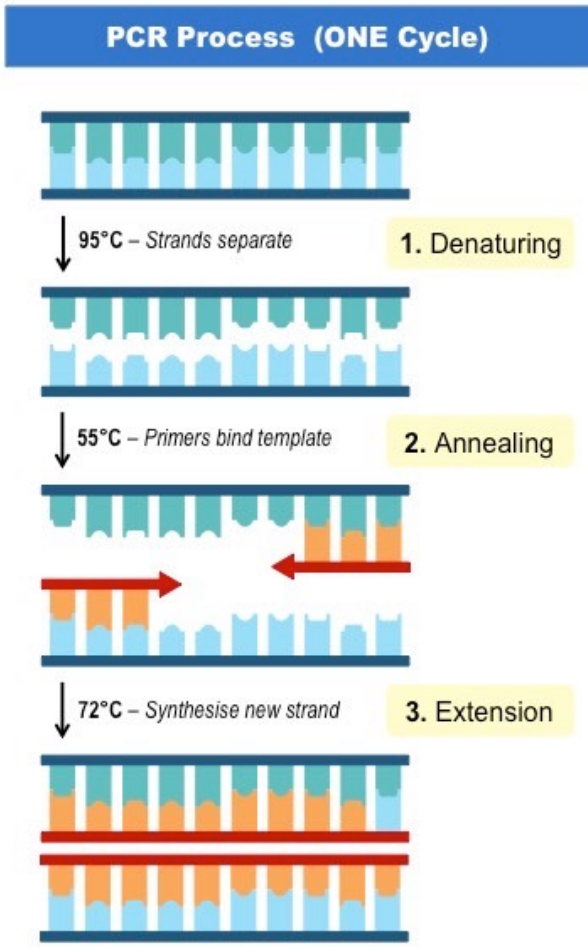
Core Microbiome Composition in Raw Wastewater

Core Microbiome Composition in Raw Wastewater

Genomic techniques for detecting pathogens in wastewater

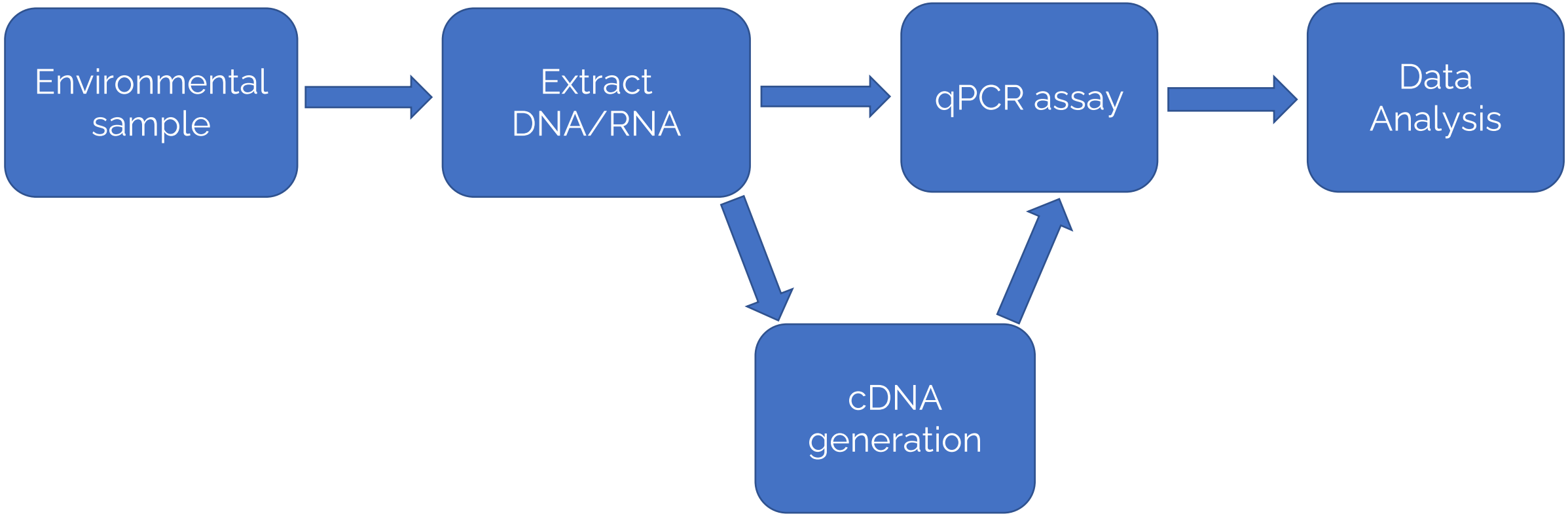




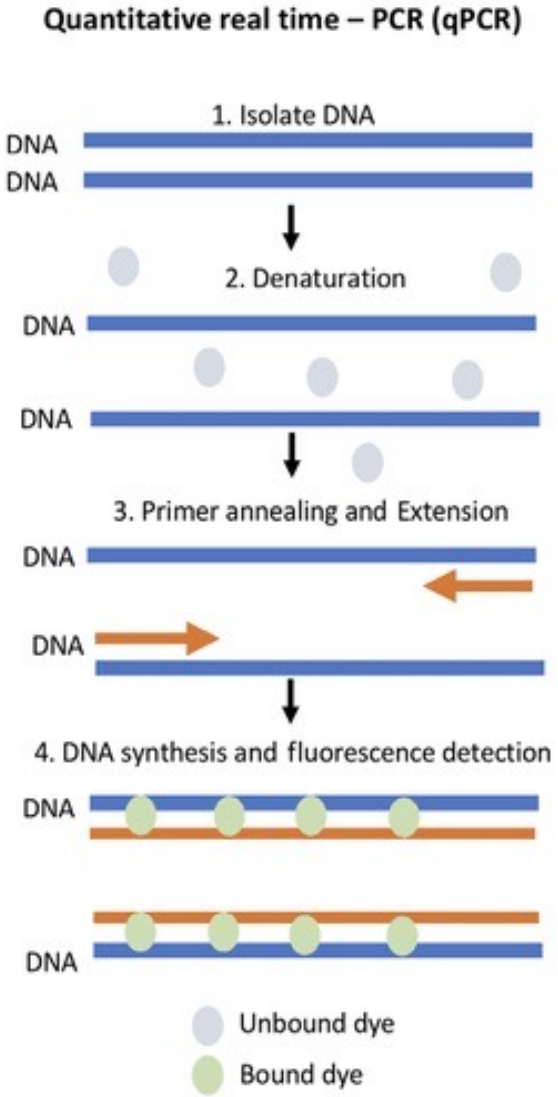


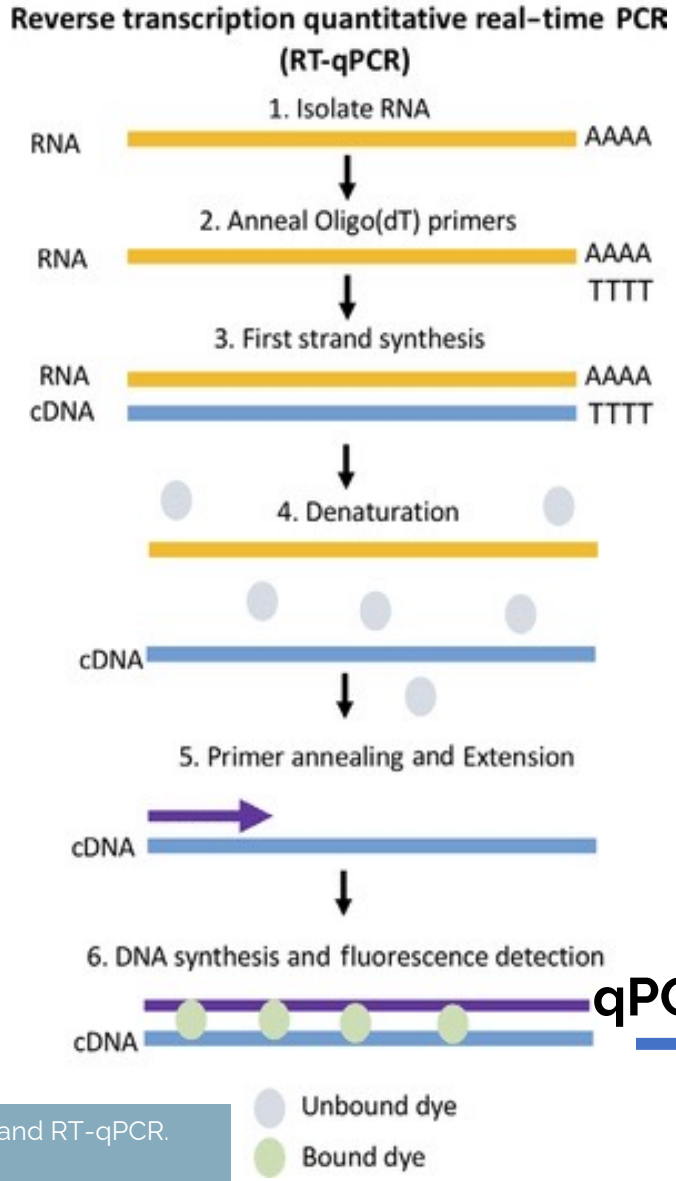
Polymerase chain reaction (PCR) technique

Workflow of Quantitative Real-Time PCR (qPCR) and Reverse Transcriptase qPCR (RT-qPCR)

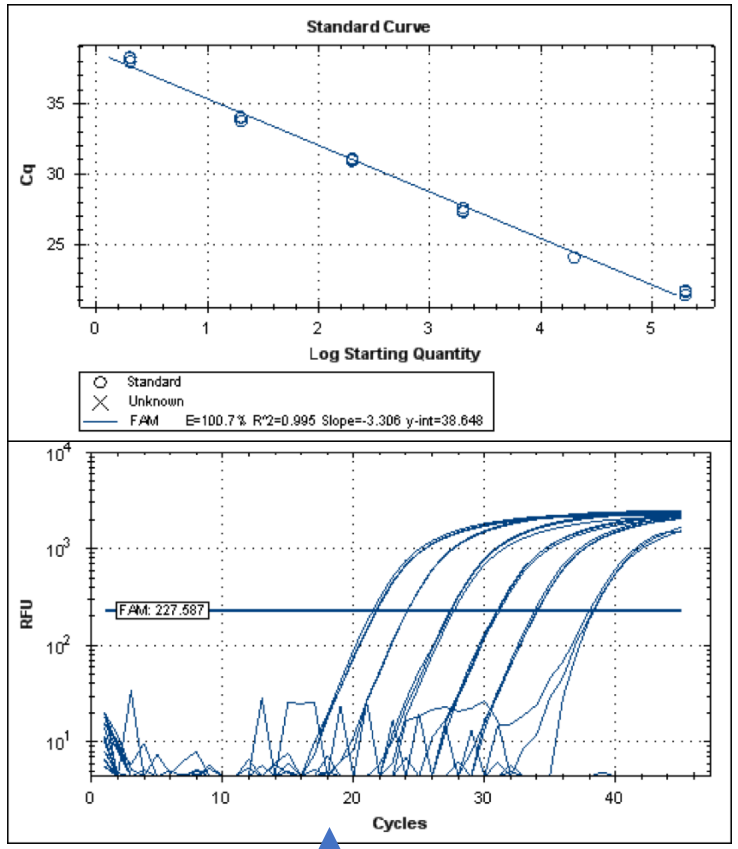


Schematic qPCR and RT-qPCR workflow





qPCR assay



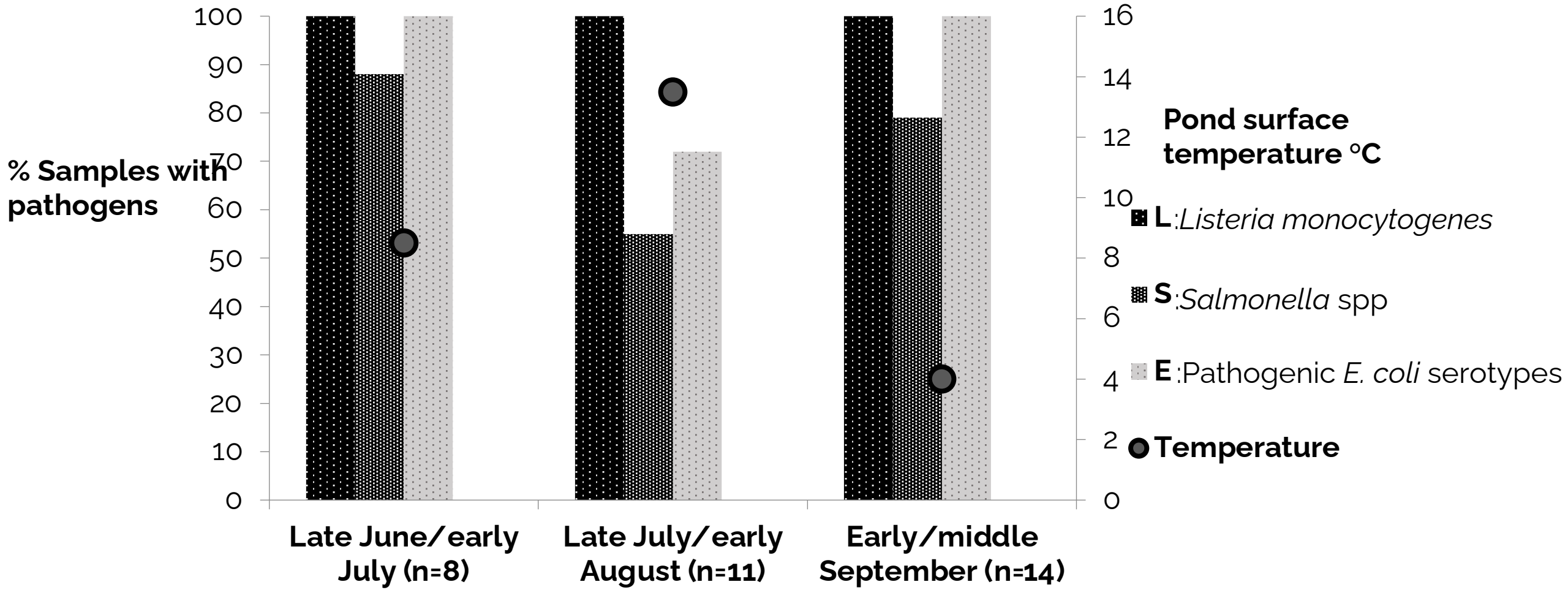
Data analysis

Source: A beginner's guide to RT-PCT, qPCR and RT-qPCR.

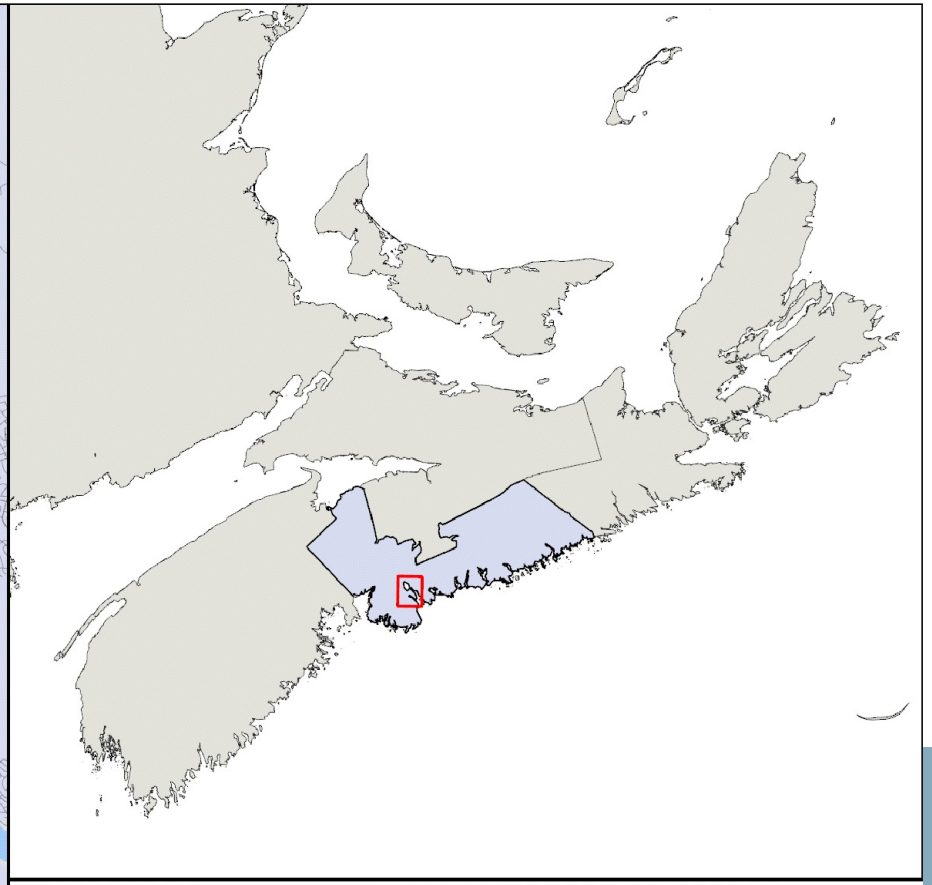
Biochem (*Lond*) (2020) 42 (3): 48-53.

https://doi.org/10.1042/BIO20200034

WSP Temperature and Removal of Pathogens in the Pond Inlet WSP



Detection of SARS-CoV-2 in wastewater in Halifax, using four RT-qPCR assays



Central Health Management Zone

Halifax Wastewater Treatment Facility

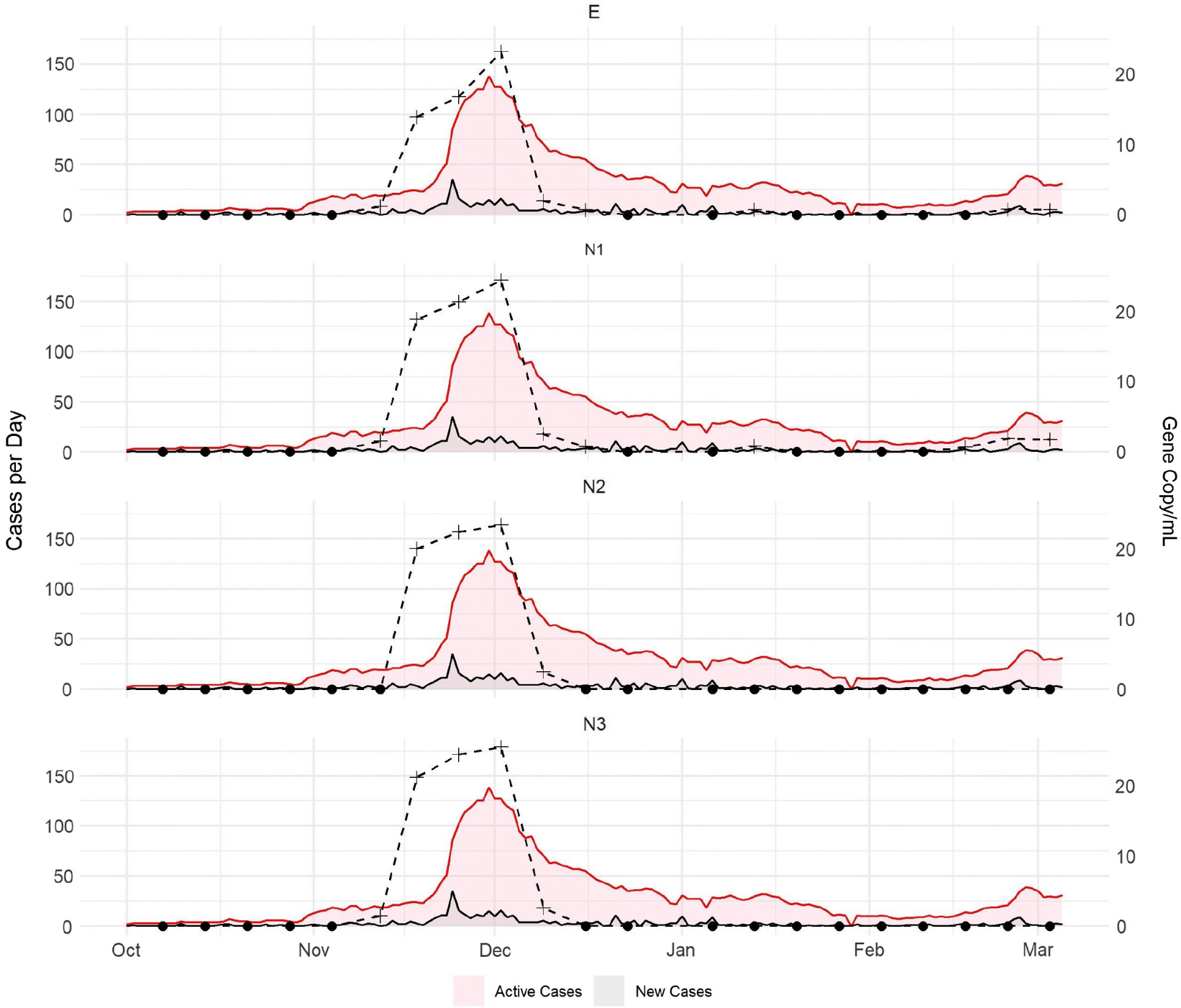
Source: Huang, Y., Johnston, L., Parra, A., Sweeney, C., Hayes, E., Truelstrup Hansen, L., Gagnon, G., Stoddart, A., Jamieson, R. (2021). Detection of SARS-CoV-2 in wastewater in Halifax, Nova Scotia, Canada, using four RT-qPCR assays. FACETS, 6, 959-965. https://doi.org/10.1139/facets-2021-0026.

- Temporal trends of the four RT-qPCR assays (N1, N2, N3, E) observed at the Halifax Wastewater Treatment Facility, and the new and active cases reported in the Central Health Management Zone (CHMZ).

- Positive signals are denoted by a + symbol.

- The N1, N3, and E assays were first detected on November 12, 2020, while the N2 was first detected on November 18, 2020.

- Both the N1 and E assays were positive on January 13, 2021.



Thank you/Nakurmiik/Qujanaq!

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